Cells of the sympathoadrenal lineage: Biological properties as donor tissue for cell-replacement therapies for Parkinson’s disease

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Abstract

Sympathoadrenal (SA) cell lineage encompasses neural crest derivatives such as sympathetic neurons, small intensely fluorescent (SIF) cells of sympathetic ganglia and adrenal medulla, and chromaffin cells of adrenal medulla and extra-adrenal paraganglia. SA autografts have been used for transplantation in Parkinson’s disease (PD) for three reasons: (i) as autologous donor tissue avoids graft rejection and the need for immunosuppressant therapy, (ii) SA cells express dopaminotrophic factors such as GNDF and TGFβ3, and (iii) although most of SA cells release noradrenaline, some of them are able to produce and release dopamine. Adrenal chromaffin cells were the first SA transplanted cells in both animal models of PD and PD patients. However, these autografts have met limited success because long-term cell survival is very poor, and this approach is no longer pursued clinically. Sympathetic neurons from the superior cervical ganglion have been also grafted in PD animal models and PD patients. Poor survival into brain parenchyma of grafted tissue is a serious disadvantage for its clinical application. However, cultured sympathetic cell grafts present a better survival rate, and they reduce the need for levodopa medication in PD patients by facilitating the conversion of exogenous levodopa. SA extra-adrenal chromaffin cells are located on paraganglia (i.e., the Zuckerkandl’s organ), and have been used for grafting in a rodent model of PD. Preliminary results indicate that long-term survival of these cells is better than for other SA cells, exerting a more prolonged restorative neurotrophic action on denervated host striatum. The ability of SA extra-adrenal cells to respond to hypoxia, differently to SA sympathetic neurons or adrenal medulla cells, could explain their good survival rate after brain transplantation.

Theme: Development and regeneration
Topic: Transplantation
Keywords: Sympathoadrenal; Chromaffin; Paraganglia; Neurodegeneration; Neurotrophic; Cathecolamine; Grafting; Parkinson’s disease; Dopamine

1. The sympathoadrenal cell lineage

Sympathoadrenal (SA) cell lineage encompasses sympathetic neurons, small intensely fluorescent (SIF) cells of sympathetic ganglia and adrenal medulla, and chromaffin cells of adrenal medulla and paraganglia. SA cells derive from a common progenitor of the neural crest (for a review, see Refs. [101,106]). The neural crest is a cell population that detaches and migrates after the closure of the embryonic neural tube, colonizing throughout the whole embryo (Fig. 1). Cells of the premigratory neural crest are multipotent and generate a wide variety of cell types (Table 1) [17,18,79]. SA progenitors derive from a population of neural crest cells that detach from the top of the neural tube and migrate throughout the embryo (Fig. 1). As neural crest cells migrate, they become developmentally restricted. SA progenitors are one of these developmentally restricted progenitor cells, which give rise
two main cell sublineages: sympathetic neurons and chromaffin cells [2]. SA progenitors express tyrosine-hydroxylase (TH), the rate-limiting enzyme in catecholamine biosynthesis, and the transcription factor Phox2 during the aggregation of neural crest cells to form the sympathetic ganglion primordia [34,107]. SA differentiation into catecholaminergic cells, which express SA1 to SA5 markers, is induced by bone morphogenetic proteins (BMP4, BMP7) produced by cells of the adjacent dorsal SA5 markers, is induced by bone morphogenetic proteins into cathecolaminergic cells, which express SA1 to sympathetic ganglion primordia [34,107]. SA differentiation into catecholaminergic cells, which express SA1 to SA5 markers, is induced by bone morphogenetic proteins (BMP4, BMP7) produced by cells of the adjacent dorsal SA5 markers, is induced by bone morphogenetic proteins into cathecolaminergic cells, which express SA1 to sympathetic ganglion primordia [34,107]. SA progenitors express tyrosine–hydroxylase (TH), the rate-limiting enzyme in catecholamine biosynthesis, and the transcription factor Phox2, which means that they synthesize either adrenaline (80% of adrenal chromaffin cells) or noradrenaline (20% of adrenal chromaffin cells) [98].

The ultimate fate of progenitor in SA lineage is modulated by environmental factors [31]. Thus, TH+ cells in the sympathetic ganglion and the adrenal gland acquire distinct phenotypes under the influence of the trophic factors FGF and CNTF produced in the ganglion environment. Mature sympathetic neurons develop from SA cells that express NGFR and respond to the NGF produced by sympathetic ganglion primordia [85]. Chromaffin cells represent a default pathway of SA cells development by the absence of environmental NGF [104–106]. Thus, SA chromaffin cells transdifferentiate ‘in vitro’ into sympathetic neuron-like cells by NGF and other neurotrophic factors (i.e., FGF or CNTF) induction [24,27,104,105,108], demonstrating that the absence of these factors – secondary to the lack of sympathetic ganglion environment – triggers the SA chromaffin cells differentiation. Moreover, SA cells differentiate into adrenal chromaffin cells under the influence of glucocorticoids, which in turn block SA neuronal transdifferentiation [107]. However, chromaffin cells do develop normally in glucocorticoid receptor deficient knockout mouse [39], although the induction of phenylethanolamine-N-methyltransferase (PNMT), the adrenaline synthesizing enzyme, is disrupted [39]. Therefore, during normal development, glucocorticoid hormones suppress SA neuronal differentiation and induce PNMT expression in chromaffin cells. In this context, although extra-adrenal chromaffin cells do not express PNMT, this enzyme can be induced by glucocorticoid treatment [13]. Whether adult chromaffin cells are able to respond, as they do ‘in vitro’, by changing their phenotype under the influence of ectopic environments, such as the brain, is being currently analyzed (see below).

1.1. Sympathetic ganglion cells

Adult SA sympathetic neurons express several cell markers (Table 2), specifically express B2 and NF (160-Kda neurofilament subunit), and almost all of them

Table 1

<table>
<thead>
<tr>
<th>Main derivatives of neural crest cells</th>
<th>Cranial crest</th>
<th>Trunk crest</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensory neurons</td>
<td>Ganglia of cranial nerves</td>
<td>Spinal ganglia</td>
</tr>
<tr>
<td>Autonomous nervous system cells</td>
<td>Enteric nervous system</td>
<td>Enteric nervous system (minor contribution)*</td>
</tr>
<tr>
<td>Endocrine and paracrine cells</td>
<td>Parasympathetic ganglia: ciliary, pterygopalatine, otic, submandibular</td>
<td>Parasympathetic ganglia: pelvic plexus</td>
</tr>
<tr>
<td>Type C cells</td>
<td>Sympathetic ganglia</td>
<td>Adrenal chromaffin medulla cells</td>
</tr>
<tr>
<td>(calcitonin-producing), type I cells of carotid body, parafollicular cells of thyroid</td>
<td>Extra-adrenal chromaffin cells</td>
<td></td>
</tr>
<tr>
<td>Pigmented cells</td>
<td>Melanocytes</td>
<td>Melanocytes</td>
</tr>
<tr>
<td>Non-neuronal cells</td>
<td>Satellite cells of ganglia, Schwann cells</td>
<td>Satellite cells of ganglia, Schwann cells</td>
</tr>
</tbody>
</table>

In bold, cells of the sympathoadrenal lineage; all derivatives of the same progenitor.

* Only serotonergic enteric neurons derive from sympathoadrenal cells [12].
are noradrenergic. Another molecules expressed by SA neurons are the intermediate filament protein peripherin [29]; nitric oxide synthase (NOS); SCG10—a 22-KDa growth-associated protein characterized in the rat superior cervical ganglia (SCG) [54]; and neural cell adhesion molecules (NCAMs). VMAT-2 is the only vesicular monoamine transporter presents in adult human sympathetic neurons, as well as in SIF cells [80]. Chromogranin A (CgA) is expressed and co-stored with catecholamines in SA neuron large dense-core vesicles [71]. Furthermore, two distinct phenotypes of SA neurons can be distinguished: (i) large type I neurons characterized by the presence of neuropeptide tyrosine (NYP), acetylcholinesterase (AChE), and neurofilament 10 (NF10); and (ii) type II neurons, smaller than type I, which possess vasoactive intestinal polypeptide (VIP) [47].

SA sympathetic neurons need neurotrophic factors, mainly NGF, and certain elements of the extracellular matrix for its maintenance, growth, and neurotransmitter expression throughout adult life [77]. These neurons express the receptor p75 and are responsive to members of the TGF-β superfamily such as BMP-2, BMP-6, and activin A that regulate catecholamine-synthesizing enzymes [78].

SA SIF sympathetic cells possess intermediate characteristics between neurons and chromaffin cells. They possess neurite-like prolongations like neurons, immunoreact for TH and DBH, and store catecholamines, similarly to chromaffin cells [32,66]. SIF cells of the SCG are able to play the role of interneurones, receiving and giving synapses, and influencing ganglionic signals. Synaptophysin and secretoneurin are two proteins that can be used as markers to identify SIF cells, whereas serotonin and TH are found in a small subpopulation of them [26]. SIF cells also express the protein gene product 9.5 (PGP9.5), neuron-specific enolase (NSE), and NCAMs (Table 2).

### 1.2. Adrenal chromaffin cells

SA adrenal chromaffin cells release catecholamines into circulation during stressful situations, eliciting a widespread alert response. The term “chromaffin” comes from their reaction with chromium salts due to the oxidation of catecholamines stored in their granules. Adrenal chromaffin cells have a rounded morphology with a mean diameter of around 15 μm, and large intracytoplasmic granules whose size distinguish adrenal cells from sympathetic neurons. There are two principal populations of adrenal cells depending of their type of catecholamine synthesizing enzymes. All adrenal medulla chromaffin cells contain TH (Fig. 2A), and the presence of DBH (noradrenaline synthesizing enzyme) or DBH plus PNMT, defines these cells as noradrenergic (NA) or adrenergic (A), respectively. In human adrenal glands, the ratio for A/NA cells is 90/10. Serotonin can be also located in PNMT containing medullary cells, suggesting that serotonin and adrenaline can be co-released from the adrenal medulla [48]. In addition, 1% of adrenal chromaffin cells contain dopamine [98].

Adrenal chromaffin cells are closely related to sympathetic neurons in terms of their bioactive substances content,
including amines, neuropeptides, and specific proteins (TH, DBH, PNMT, NCAMs, and SNAP-25) and, like sympathetic neurons, receive preganglionic cholinergic, aminergic, and peptidergic neuronal inputs. Adrenal medulla cells also express several trophic factors such as FGFs, TGF-\(\text{h}\), GDNF, and interleukines, which have been proved to promote survival of cultured peripheral and central nervous system neurons [56,65,97,103] (Table 2). Other proteins present in adrenal chromaffin cells are the members of the chromogranin family CgA, CgB, and secretogranin II (SgII) [98] (Table 2). Noradrenergic chromaffin cells exhibit three markers that they do not share with adrenergic chromaffin cells: GAP43, the adhesion molecule L1, and VMAT-2 [28]. Fetal adrenal chromaffin cells in the rat are hypoxia-sensitive before being innervated by sympathetic fibers. Hypoxia-mediated catecholamine release during parturition plays a key role for the neonatal transition from intrauterine to extrauterine life [83,84]. This developmentally regulated O\(\text{2}\)-sensing mechanism seems to be lost during postnatal life and is absent in adult chromaffin cells [82,83].

1.3. Extra-adrenal chromaffin cells

Extra-adrenal paraganglia are located adjacent to organs near the adrenal gland (mainly kidneys), on the abdominal sympathetic region (solar plexus), next to the genitals glands, and on the low abdominal aorta—the Zuckerkanld’s organ (ZO). Paraganglia are constituted by mesenchyma and chromaffin cells, although scattered Schwann’s cells and connective tissue can be also found. Chromaffin cells aggregate in fascicles surrounded by mesenchyma, with the appearance of “cell nests” on coronal sections. This arrangement of chromaffin cells was originally described by Kohn in 1903, who named fascicles and nests as “Zellsträngen” and “Zellballen”, respectively ([52], cited in Refs. [94,95]). Paraganglionic chromaffin cells have a rounded morphology, with a diameter ranging from 15 to 20 \(\mu\)m, with many catecholamine containing intracytoplasmic granules with variable diameter (60–400 nm). As SA paraganglionic cells developed outside of the adrenal gland (see above), NA is their main neurotransmitter, representing the 90% of the total catecholamine content, and most chromaffin cells express DBH [13,26,27,94] (Fig. 2 B). Paraganglia SA cells also possess several peptides, chromogranins, and trophic factors, which constitute the so-called “cocktail” secretion of paraganglia [94,102,103,110] (Table 2).

The paraganglia are a second source (the main one is the adrenal medulla) of circulant cathecolamines, which are released into blood in response to chemical stimuli (mainly on stressful situations). Unlike adrenal medulla, paraganglia SA cells are poorly innervated [45], and they mostly respond to chemical rather than to synaptic signals [45,94,95]. This characteristic could be useful in grafting experiments in which synaptic integration between host and SA-grafted cells are absent. Thus, environmental signals (i.e., hypoxia) could elicit SA cells secretion of their products without synaptic contacts (see below) [46].

ZO is the biggest extra-adrenal paraganglion, and was described by Emil Zuckerkanld in 1901 [112]. In mammals, ZO is located between the emergence of the inferior mesenteric and iliac arteries [1,91,112], the ZO being critical for the normal development of the cardiovascular system [92,93]. In humans, there are two ZO (8–15 mm in length), even though the presence of small accessories ones has also been reported [91]. There is usually one ZO in rats (5–8 mm in length), although two or more accessories smaller paraganglia can be also found [37]. ZO chromaffin cells react with potassium dichromate (classical Orth’s reaction) and contain CgA. ZO cells also express TH and DBH but lack PNMT [13,90,95] (Table 2).
2. Transplantation of sympathoadrenal cells

The ideal donor tissue for transplantation is the own patient tissue, which avoids graft rejection and the need for immunosuppressant therapy. In this way, almost all SA cell types have been transplanted in animal models of PD, and some of them have been also tested in human as autologous cell-replacement therapy for PD (Table 3). PD is caused by the massive degeneration of the dopamine nigrostriatal system leading to a strong deficit of dopamine neurotransmission in the striatum. Therapeutic effect exerted by grafted SA cells in PD acts in two ways: (i) an important restorative neurotrophic action on damaged host dopaminergic tissue through the delivery of dopaminotrophic factors such as GDNF and TGF-βs, which protect dopaminergic neurons from degeneration [9,43,51,56,65,97,99,103]; and (ii) a partial recovery of dopamine levels by the release from the graft of a certain amount of dopamine (mainly adrenal cells), even though only minute amount of dopamine are released from most SA cells [33,61,67,76]. The greatest handicap of using SA cells for grafting is their poor survival in the brain, even though some types of SA cell grafts appear to present a better survival rate, as it is discussed below.

2.1. Transplantation of sympathetic neurons

Several research groups have grafted SCG neurons in animal models of PD with widely varying results. SCG neurons release NA and express “dopaminotrophic” factors and other neuroprotective agents such as neuropeptides and cytokines [102]. Stenevi et al. [88] reported a very poor survival of intrastriatally transplanted sympathetic neurons, except for those grafts placed near the choroidal fissure. In these cases the revascularization of the transplant from the choroid plexus vessels enhances graft survival. Thus, the close relationship between graft survival and an efficient revascularization from the surrounding tissue strongly suggests that adequate oxygen supply is a key factor for graft survival. Grafted cells next to the choroidal plexus give rise to numerous fibers or neurites spreading into host brain. This interesting phenomenon has been frequently observed in grafted sympathetic SA cells and other neural-crest-derived cells (i.e., carotid body cells) and opens the possibility that host striatum neurons and grafted cells could establish synaptic contacts that might regulate graft dopamine release. However, in contrast to transplanted fetal dopaminergic neurons [30,64], the establishment of host-graft synapses has not been yet demonstrated in sympathetic SA cell grafts.

Transplanted sympathetic neurons into the host striatum far away from the choroidal plexus fail to survive, and even NGF preincubation of grafted neurons is unable to improve cell survival [88]. Thus, the main limitation of SCG cells graft into adult brain is the massive cell death after transplantation into the striatum [73]. This handicap seems to have been solved by using cultured sympathetic neurons for grafting. In this case, grafts’ long-term survival depends on the time of maintaining cells in culture, being maximal with 2-week cultured cells [68]. Grafts of these cultured cells significantly reduced the rotational behavior after apomorphine administration 12 weeks after transplantation [68]. Because of the apparently beneficial effects of cultured sympathetic cells in animal PD models, autologous SCG transplantation in PD patients has been tested [69]. In five grafted PD patients the on-period induced by levodopa was improved [69]. Although SCG-grafted cells are noradrenergic, their beneficial effect could be explained by their capability to convert exogenous levodopa to dopamine [69,70]. Thus, cultured sympathetic neurons grafts induce a partial symptomatic relief in PD patients and reduce the need for levodopa medication. Therefore, sympathetic ganglia neurons can be used as donor tissue for autologous

<table>
<thead>
<tr>
<th>Type of cell</th>
<th>Dopamine secretion</th>
<th>Neurite outgrowth from graft</th>
<th>Trophic striatal reinnervation</th>
<th>Long-term survival rate</th>
<th>Hypoxia resistance</th>
<th>Clinical efficacy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fetal mesencephalic cells</td>
<td>Yes</td>
<td>Yes, abundant</td>
<td>Scarce</td>
<td>3–5%</td>
<td>No</td>
<td>Acceptable*</td>
</tr>
<tr>
<td>Adrenal chromaffin cells</td>
<td>Minute</td>
<td>Low</td>
<td>Yes</td>
<td>0%</td>
<td>No</td>
<td>Poor and limited</td>
</tr>
<tr>
<td>Sympathetic neurons</td>
<td>Minute</td>
<td>Yes (non-cultured cells)</td>
<td>Scarce</td>
<td>0% (solid tissue) 1–3% (2-week cultured cells)</td>
<td>No</td>
<td>Partial, reduce the need for levodopa medication [70]</td>
</tr>
<tr>
<td>Extra-adrenal chromaffin cells</td>
<td>Minute</td>
<td>Low</td>
<td>Yes</td>
<td>10–15%</td>
<td>Yes</td>
<td>Non-tested</td>
</tr>
<tr>
<td>Stem cell-derived dopamine cells</td>
<td>Yes</td>
<td>Unknown</td>
<td>Scarce</td>
<td>Yes, but not quantified</td>
<td>No</td>
<td>Non-tested</td>
</tr>
<tr>
<td>Carotid body glomus cells</td>
<td>Yes</td>
<td>Low</td>
<td>Yes</td>
<td>Highb</td>
<td>Yes</td>
<td>Limitedc</td>
</tr>
</tbody>
</table>

*a* Some disadvantages are the difficulty in obtaining sufficient viable embryonic mesencephalic tissue, and that some patients have been reported to present disabling dyskinesias [42].

*b* Long-term graft survival has been reported in most of transplanted animals, without estimating the number of surviving grafted cells [96].

*c* Limited efficacy of autologous transplants has been explained by PD-related deterioration of old carotid body glomus cells [3].
transplantation in PD, but further investigation is required to improve cell survival, the main disadvantage for the clinical application of this cell substitution technique.

2.2. Transplantation of adrenal chromaffin cells

Adrenal chromaffin cells were the first non-neuronal cells grafted in animal models of PD. From a clinical point of view, they have the advantage that can be obtained from one of the patient’s own adrenal glands. Autologous adrenal cells transplanted into the denervated striatum exerted some beneficial effects in animal models of PD [6,13], and this was also the case for the first reported grafting in PD patients [63]. Since the proportion of dopaminergic cells is very low in transplanted donor tissue – only 1% of the entire adrenal chromaffin cells population releases dopamine – the graft functional effects could not be exclusively accounted for by dopamine release. In fact, as it is the case of other members of the SA cell lineage, the neurotrophic effect of chromaffin cells was proposed as the responsible for PD symptoms amelioration [33]. However, the initial good results were not confirmed by other authors, and it is currently accepted that survival of adrenal medulla grafts is low in animal models of PD [41], and extremely low 6 months after grafting in PD patients [44], which show only transient functional amelioration [58,74]. Histological studies of long-term grafts experiments give the explanation. Grafts became necrotic and were surrounded by a perigraft halo [7,14,16] of microglia and macrophages which persist for many months after grafting, limiting thus the trophic action of the grafts [8]. Therefore, long-term survival and functional efficacy of adrenal chromaffin cells grafts are very poor in the brain, either in experimental PD models or in PD patients, and this approach is no longer pursued clinically (Table 3).

2.3. Transplantation of extra-adrenal chromaffin cells

Extra-adrenal chromaffin cells are currently used for grafting in animal model of PD in our laboratory [35,36,38]. ZO can be easily removed from its location [37], and preliminary results indicate that after ZO transplantation parkinsonian rats (6-OHDA model) showed a long-term behavioral improvement manifested by a progressive and sustained reduction of several motor and sensorimotor parkinsonian deficits [35,37]. These functional effects were related to survival of around 10–15% of grafted cells 3 months after grafting, a remarkable finding that indicates that ZO-grafted cells survived for a long period of time. The ectopic placement of ZO within brain parenchyma did not induce changes in the ZO cell phenotype, which differently to other grafted neural-crest derived cells (i.e., carotid body cells [36]) did not develop neurite-like prolongations. Immunohistochemical analyses revealed the presence of TH+, DBH+, and CgA+ cells inside grafts [38]. TH+ density of host striatum was significantly high after grafting (Figs. 3B–C) and accompanied by a reliable increase (even though two times lower than that of naïve control animals) of striatal dopamine content [37]. Hence, this partial dopamine levels restitution after ZO grafting explain the behavioral improvements obtained in parkinsonian rats, which are directly related with the recovery of the

Fig. 3. (A) Coronal section through the mesencephalon of a rat injected with 6-OHDA into the right substantia nigra. Ten days after 6-OHDA treatment, injection of the fluorescent tracer fluorogold in both striata (signal of injection in the right striatum is shown in the inset) retrogradely labels striatum projecting neurons of the damaged right substantia nigra (r) and non-affected left substantia nigra (l). (B and C) Coronal sections through the forebrain of ZO-grafted (B) and sham-grafted parkinsonian rats (C). A net TH-positive signal (reinnervated area) is evident in ZO-grafted right striatum (B, asterisk), while the right striatum of sham-grafted rats lacks TH immunostaining (C, compare with the non-affected contralateral left striatum). White line marks the mesencephalon midline in A.
dopaminergic tone of dorsal striatum [10,21,22]. However, the increase of striatal dopamine content after grafting cannot be explained by dopamine ZO cells release because grafted cells were noradrenergic (Table 2), and only minute amounts of dopamine can be released from extra-adrenal noradrenergic chromaffin cells [61,67,76]. Noradrenaline (NA) balance is also altered in PD and the deficit of the noradrenergic system could play a role on its pathophysiology [15, 87], suggesting that NA released by grafted ZO cells would exert some beneficial effects on parkinsonian rats. However, functional recovery of parkinsonian rats after ZO grafts could not be explained by the sole effect of NA release from grafted cells.

The recovery of the dopaminergic tone after grafting can be related to the striatal reinnervation by the sprouting of spared host dopaminergic fibers. ZO-grafted cells express GDNF and TGF-β1, and significant levels of these neurotrophic factors have been detected in the striatal tissue [37]. GDNF and TGF-β1 protect dopaminergic neurons from degeneration [9,43,51,56,65,97,99,103]. Hence, the neurotrophic action of GDNF and TGF-β1 secreted by ZO cells could induce the sprouting of nigrostriatal remaining axons, leading to the host striatal reinnervation (Figs. 3B–C). Furthermore, differently from adrenal medulla or sympathetic ganglia cells, extra-adrenal chromaffin cells express the hypoxia-inducible factor EPAS-1 [38]. This transcription factor is present in diverse cells under normoxic conditions and it is up-regulated under hypoxic conditions [5,92,109]. Therefore, it could be hypothesized that like the oxygen-sensitive carotid body cells [40], ZO chromaffin cells respond to hypoxia [46,94] and are able to survive under the hypoxic environment of the brain [5,96]. Furthermore, the lack of EPAS-1 could explain why adrenal medulla chromaffin cells or sympathetic neurons show a poor survival after intrabrain transplantation, leading to grafting failure. Thus, the main advantage of ZO grafts is the long survival of their extra-adrenal chromaffin cells, which allow them to exert a chronic dopaminotrophic action based on the delivery of GDNF and TGF-β1, and likely other neuroprotective agents [102]. However, it should be noted that the work on this type of transplant is preliminary and further basic studies are needed before testing its clinical applicability. For instance, since autografts are expected to be carried out in elderly population suffering from PD, more animal experimental studies on the efficacy and survival of old ZO cells after grafting are needed.

3. Comparisons among transplants of sympathoadrenal cells and other cell grafts

Grafting procedures performed as clinical therapy for PD could be classified in two main groups: (i) grafts devoted to the restoration of the dopamine levels in the denervated striatum by using dopamine-secreting cells (i.e., fetal or embryonic dopaminergic cells, engineered stem cells, etc.; for a review, see Ref. [4]), or cultured sympathetic neurons that convert exogenous levodopa in dopamine [68–70], and (ii) dopaminotrophic grafting procedures directed to provide a trophic support for the remaining host nigrostriatal dopaminergic neurons, in order to stop neuronal death or to restore the striatal dopaminergic circuit. Since the eighties, grafting procedures have been extensively tested in animal models of PD, before their clinical application [6,10,14,21,41,54,60,61,71]. One of the most widely used animal models of PD consists in the neurotoxic destruction of the dopaminergic neurons of the substantia nigra by 6-hydroxydopamine (6-OHDA) administration. This animal model parallels human disorder well and produces several parkinsonian deficits such as akinesia, and reproducible spontaneous and drug-induced turning behavior [23,81,100]. Early studies demonstrated the specificity of 6-OHDA neurotoxic action [50], which induced a complete neuronal cell loss of 6-OHDA injected substantia nigra [86]. However, evidence are accruing that in most cases the substantia nigra is only partially damaged and some dopamine nigrostriatal projecting neurons still survive after 6-OHDA treatment. Fig. 3 exemplifies this case: injection of fluorogold into the striatum of a 6-OHDA-treated rat, with a strong parkinsonian asymmetry (more than 700 turns per hour), labels several striatal projecting neurons of the substantia nigra pars compacta. Therefore, it could be hypothesized that the recovery of PD motor symptoms after grafting could depend on the ability of undamaged axons to react to the neurotrophic influence of the graft apart from dopamine release from dopamine-secreting transplants. Why nigrostriatal axons respond better to the trophic influence in some animals than in others, under the same experimental conditions, remains already unknown.

SA chromaffin cells are mostly noradrenergic (Table 2) [45,94,98], and only minute amounts of dopamine can be released by non-dopaminergic chromaffin cells [61,67,76]. Therefore, the functional effects of these types of grafts in PD models cannot be explained by dopamine secretion. This is an important difference with other types of grafts based on dopamine delivery such as transplants of embryonic dopamine neurons of the substantia nigra, the most efficient cell graft tested so far (Table 3). Fetal nigral cell grafts restore the dopamine levels in two ways: (i) releasing dopamine, and (ii) inducing host striatal reinnervation through neurites arising from grafted cells, thereby leading to the restoration of the dopaminergic neurotransmission [33]. Fetal nigral cells survival rate is low, and only 3–5% of grafted neurons survive after grafting [19,89], but enough to induce amelioration and tissue reinnervation. Moreover, grafted neurons integrate with the host striatum and axodendritic synapses between host and graft have been found, suggesting that dopamine release can be regulated, a fact that could account for good functional effects [53].Clinical trials have shown that mesencephalic dopamine neurons obtained from human embryo cadavers survive and function after grafting in the striatum of PD patients [57,72].
The grafts are able to normalize striatal dopamine release, and some patients have been able to withdraw from levodopa treatment for several years and resume an independent life [33,53,75]. Apart from ethical, practical, and safety issues associated with the use of tissue derived from aborted human fetuses [20,33], an important limiting factor for this procedure is that several embryos are needed to obtain sufficient amount of tissue because of the low survival rate of grafted neurons [33,49,57,72]. Besides, since autologous tissue cannot be used as donor for transplantation, immunosuppressant therapy is needed for avoiding graft rejection. Furthermore, in the recent Denver/New York study, severe disabling dyskinesias were reported as side effect in a significant number of PD-grafted patients [42]. This side-effect seems to be caused by unbalanced partial recovery of dopamine signal in different transplanted areas of both putamina [62]. Several authors have suggested that dyskinetic effects could be overcome by improving cell culture procedures and carefully selection of mesencephalic dopamine cells [49].

Similar results to those found with fetal nigral neurons in preclinical studies have been obtained with stem cells. Neurons with dopaminergic phenotype have been generated in culture from mouse and monkey embryonic stem cells (ESCs) and from rodent and human fetal neural stem cells (NSCs) [55,59,89]. Transplanted NSCs survive and integrate into host brain after intrastriatal transplantation [111]. Moreover, Isacson and collaborators demonstrated that ESCs implanted in dopamine-depleted striatum function as replicas of the dopaminergic neurons lost in PD and can restore some motor deficits in animal models of PD [11,49]. Although further experiments are needed, stem cell therapy appears to be a good candidate for transplantation in PD and could overcome the problem of the low survival rate of fetal neurons.

The main advantage of SA cells for grafting is that, as autologous tissue, graft rejection and the need for immunosuppressant therapy are avoided, despite the fact that they do not act as dopamine secreting ‘minipumps’. Functional effects in PD patients appear to be caused by the dopaminotrophic action in the striatum leading to improvement of dopaminergic tone and, in the case of sympathetic neurons, the capability for converting exogenous levodopa to dopamine [69,70]. Extensive striatal reinnervation characterized by abundant TH+ fibers running within the striatum has been observed after SA cell grafts when compared with PD non-grafted rats (Figs. 3B–C). This reinnervation arises from remaining undamaged nigrostriatal dopaminergic fibers, because a recovery (even partial) of dopamine neurons of damaged substantia nigra has not been undoubtedly demonstrated with neither type of graft. Striatal reinnervation is more widespread with SA cells grafts than with transplants of fetal nigral dopamine neurons, where it is mostly dependent of the neurite outgrowth of grafted neurons and where a limited number of afferents from host neurons to transplanted cells have been detected [30,53]. It must be considered that degeneration of the host striatogniral system continues in PD after every type of graft tested so far. Hence, striatal trophic regeneration would help reconstitute a neuronal network capable of restoring feedback-controlled release of dopamine in the nigrostriatal system. Long-term trophic action appears to be precluded by the fact that both sympathetic neurons and adrenal chromaffin cells show a very poor survival into the striatum. This is a serious disadvantage for the clinical application of this cell substitution therapy.

Recent findings indicate that extra-adrenal ZO cells have a good survival rate after grafting, a phenomenon that could be associated to resistance of SA extra-adrenal cells to hypoxia in comparison with other SA lineage cells whose survival rate is low [35,37]. The biochemical basis of hypoxia resistance of extra-adrenal cells as well as their effects on graft efficacy are currently being explored, in order to give experimental support for the clinical applicability of extra-adrenal chromaffin cells. In this context, grafts of carotid body cells, another type of neural-crest derived cells, have proved to be effective in ameliorating functional deficits in PD rodent and primate models [36,60,96], and it is known that glomus cells are highly resistant to hypoxia—carotid body cells behave as oxygensensing elements capable of supporting low oxygen tension [40]. This fact would explain the high proportion of grafted glomus cells that survive long time after transplantation [36,96]. Functional amelioration following carotid body cells grafting was explained by the GDNF trophic action rather than by dopamine secretion [96], in the same way that the trophic effects attributed to SA cell grafts. However, the only clinical study available revealed a limited functional amelioration in PD patients grafted with autologous carotid body glomus cells, likely explained by a PD-related deterioration of old carotid body glomus cells. Furthermore, old carotid body cells degenerate within the first 3 months after grafting [4], probably due to the loss of hypoxia responsivity properties of aged carotid body cells [25].

4. Conclusion

The SA cells lineage gives two main cell sublineages: sympathetic cells (neurons and SIF cells) and chromaffin cells (adrenal and extra-adrenal cells). These ‘adrenergic’ cells express trophic factors that promote survival of dopamine neurons ‘in vitro’, and protect dopamine neurons ‘in vivo’. An important current area of medical research is devoted to the development of cell-replacement therapies for PD. Fetal dopaminergic neurons and engineered stem cell grafts appear suitable and promise future strategies. However, SA cells also appear as a putative cell source for autografting procedures in order to avoid heterograft inconveniences. Cultured sympathetic neuron grafts possess limited efficacy in clinical trials, and they mostly act...
facilitating the conversion of dopamine from exogenous levodopa, suggesting that this type of graft could be useful for reducing the need for levodopa medication in PD patients. Extra-adrenal chromaffin cells have been tested in rats’ model of PD, where they showed a better survival rate than adrenal chromaffin cells. Long-term cell survival allows a sustained neurorestorative action, stimulating the sprouting of undamaged host nigrostriatal axons. This good survival of grafted SA extra-adrenal cells could be related to their ability to respond to hypoxia, differently to SA sympathetic neurons or adrenal medulla cells. However, more preclinical studies are warranted before testing its clinical applicability.

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